

EXTENDED REPORT

# Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups

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**ABSTRACT**

**Objectives** The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of rare autoimmune diseases characterised by muscle weakness and extramuscular manifestations such as skin rashes and interstitial lung disease. We genotyped 2566 IIM cases of Caucasian descent using the Immunochip; a custom array covering 186 established autoimmune susceptibility loci. The cohort was predominantly comprised of patients with dermatomyositis (DM, n=879), juvenile DM (JDM, n=481), polymyositis (PM, n=931) and inclusion body myositis (n=252) collected from 14 countries through the Myositis Genetics Consortium.

**Results** The human leucocyte antigen (HLA) and *PTPN22* regions reached genome-wide significance ( $p < 5 \times 10^{-8}$ ). Nine regions were associated at a significance level of  $p < 2.25 \times 10^{-5}$ , including *UBE2L3*, *CD28* and *TRAF6*, with evidence of independent effects within *STAT4*. Analysis of clinical subgroups revealed distinct differences between PM, and DM and JDM. *PTPN22* was associated at genome-wide significance with PM, but not DM and JDM, suggesting this effect is driven by PM. Additional suggestive associations including *IL18R1* and *RGS1* in PM and *GSDMB* in DM were identified. HLA imputation confirmed that alleles *HLA-DRB1\*03:01* and *HLA-B\*08:01* of the 8.1 ancestral haplotype (8.1AH) are most strongly associated with IIM, and provides evidence that amino acids within the HLA, such as *HLA-DQB1* position 57 in DM, may explain part of the risk in this locus. Associations with alleles outside the 8.1AH reveal differences between PM, DM and JDM.

**Conclusions** This work represents the largest IIM genetic study to date, reveals new insights into the genetic architecture of these rare diseases and suggests different predominating pathophysiology in different clinical subgroups.

**INTRODUCTION**

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of rare autoimmune diseases, the major phenotypes of which are dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM) and DM/PM overlapping with other connective tissue diseases.<sup>1</sup> IIMs are primarily characterised by the presence of proximal muscle weakness, elevated levels of skeletal muscle enzymes and inflammatory infiltrates in skeletal muscle, but may also present with extramuscular manifestations including skin rashes, interstitial lung disease and malignancy that often correlate with serum antibody status.<sup>2</sup>

IIMs are thought to be complex genetic diseases, initiated by immune activation following specific environmental events in genetically predisposed individuals. The strongest genetic association in the IIM has been consistently within the major histocompatibility complex (MHC),<sup>3</sup> specifically with the 8.1 ancestral haplotype (8.1 AH). A recent genome-wide association study in DM, and a candidate gene study, also indicate overlap of genes implicated in other autoimmune diseases.<sup>4 5</sup> The Immunochip is a custom-designed array containing coverage of 186 established autoimmune susceptibility loci and extended coverage across the MHC.<sup>6</sup> In this study, we report an Immunochip analysis of 2566 global IIM cases and 15 651 controls, representing the largest genetic association study to date in IIM.

**METHODS**

**Samples**

Two thousand nine hundred and fifty-four samples from 14 countries were collected through the

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Myositis Genetics Consortium, and written informed consent was obtained from all cases. There is overlap between these samples and previous IIM genetic studies.<sup>3–5</sup> IIM cases were included if they fulfilled probable or definite Bohan and Peter classification criteria for PM, juvenile PM, DM or juvenile DM (JDM),<sup>7, 8</sup> and Griggs or European Neuromuscular Centre or Medical Research Council criteria for IBM.<sup>9–11</sup> Eleven samples met the criteria for antisynthetase syndrome,<sup>12</sup> however available clinical data was not able to differentiate between PM or DM. These were included in the combined IIM analysis, but removed from the clinical subgroup analysis.

ImmunoChip control data from 12 countries was provided by four disease consortia (see online supplementary methods).

### Genotyping and quality control

Genotyping was performed in accordance with Illumina's protocols in the UK (Centre for Genetics and Genomics Arthritis Research UK, University of Manchester, UK) and the USA (Feinstein Institute, New York, New York, USA). Standard sample and SNP quality control (QC) was performed in PLINK V1.07 (see online supplementary methods).

### Statistical analysis

Statistical analysis was performed in PLINK V1.07 using a logistic regression applying an additive model, including the top 10 principal components as covariates to control for population stratification. Evidence for additional independent effects was investigated using a stepwise logistic regression including the most associated variants as covariates in subsequent conditional analyses.

### Functional annotation

Associated loci were interrogated for potentially causative variants using expression quantitative trait loci (eQTL) databases, and the functional prediction tools PolyPhen-2,<sup>13</sup> SIFT<sup>14</sup> and phastCons17-way<sup>15</sup> (see online supplementary methods).

### MHC imputation and association analysis

Classical human leucocyte antigen (*HLA*) alleles and corresponding amino acid sequences were imputed using SNP2HLA. A logistic regression assuming an additive model was used to test for association, and forward stepwise logistic regression was used to test for independent effects (see online supplementary methods). Classical four-digit HLA alleles were preferentially reported, unless an amino acid association explained more risk than HLA alleles alone.

## RESULTS

### Genotyping quality control

After stringent SNP and sample quality control we analysed 90 536 genetic variants in 2566 IIM cases and 15 651 controls of Caucasian descent (table 1). A breakdown of this cohort by clinical subgroup is reported in online supplementary table S1. Australia, Denmark and Switzerland did not have an ethnically matched control group; however, these were adequately matched by existing cohorts (UK, Sweden and Germany, respectively). By including the top 10 principal components as covariates and calculating the genomic inflation on a set of null SNPs (from a study investigating the genetic basis for reading and writing ability)<sup>16</sup> on the ImmunoChip gave a  $\lambda_{GC1000}$ =1.05, indicating that cases and controls are well matched for ethnicity (see online supplementary figure S1).

**Table 1** Number of idiopathic inflammatory myopathy (IIM) cases included in the analysis

Number of Cases		Number of Controls	
Australia	120	–	
Belgium	12	Belgium	351
Czech Republic	236	Poland	526
Denmark	53	–	
France	37	France	497
Hungary	209	Hungary	257
		Germany	1029
Italy	37	Italy	969
		Italy (RAF cohort)	813
Netherlands	38	Netherlands	2020
Norway	63	Norway	730
Sweden	269	Sweden	1938
Spain	73	Spain	409
Switzerland	3	–	
UK	993	UK	4332
USA	423	USA	1780
Total	2566	Total	15 651

Number of IIM cases after QC, by country of origin. Control samples shared from ImmunoChip consortia matched by closest ethnicity. RAF, Independent Italian cohort from Ospedale San Raffaele.

### *HLA* and *PTPN22* are the most strongly associated regions in IIM

Two regions in this study reached genome-wide significance ( $p < 5 \times 10^{-8}$ ) (figure 1A and table 2). As expected, the most strongly associated region was within the MHC ( $p = 9 \times 10^{-133}$ ) (see online supplementary figure S2). *HLA*-imputation was performed separately on this locus.

The other region reaching genome-wide significance was within the *PTPN22* locus (rs2476601;  $p = 7.22 \times 10^{-9}$ ), an established autoimmune risk locus. This SNP/locus has been previously associated in an IIM candidate gene study,<sup>3</sup> but was not associated in a genome-wide association study in DM.<sup>4</sup>

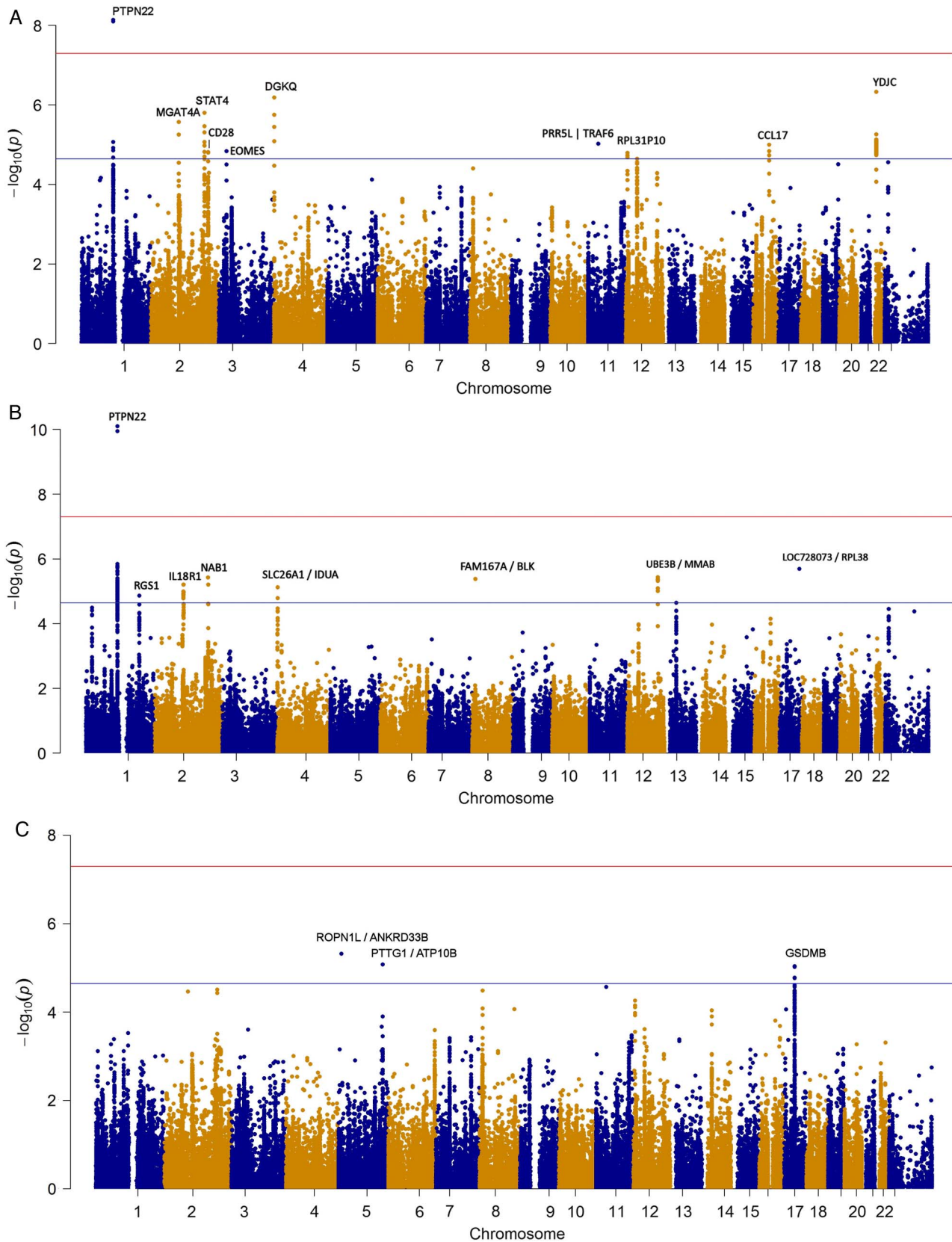
### A further nine regions were associated at a suggestive level of significance

We next investigated associations reaching our suggestive tier of association ( $p = 2.25 \times 10^{-5}$ ) calculated using the genetic Type 1 error calculator.<sup>31</sup> This estimates the effective number of independent tests based on the linkage disequilibrium (LD) between SNPs contained on the genotyping array. Here, we found evidence of a further nine associated loci (table 2).

The third most strongly associated SNP in this analysis was in the *YDJC* gene (rs5754467) on chromosome 22 ( $4.67 \times 10^{-7}$ ). This SNP tags a large haplotype block containing *UBE2L3*, an established autoimmune risk locus.

*STAT4* is a susceptibility locus for many autoimmune diseases. The lead SNP in this region was protective, which is a novel finding in IIM (rs4853540,  $p = 1.57 \times 10^{-6}$ ). Stepwise logistic regression analysis in this region suggested an independent risk effect of rs10174238 ( $p = 1.08 \times 10^{-5}$ , OR=1.17, 95% CI 1.09 to 1.26) (see online supplementary figure S3) and a further potential independent effect was seen at rs932169 ( $p = 2.88 \times 10^{-5}$ , OR=1.25, 95% CI 1.13 to 1.39).

Further variants reaching our suggestive significance threshold reveal loci of interest that have been previously associated with autoimmune disease, including *DGKQ*, *EOMES*, *CD28* and *PRR5L/TRAF6*. Associated SNPs that tag risk haplotypes



**Figure 1** Manhattan plots of the IIM, PM and DM+JDM analyses, with the MHC region removed. The red and blue lines represent genome-wide level of significance ( $p=5\times 10^{-8}$ ) and suggestive significance ( $p=2.25\times 10^{-5}$ ), respectively. (A) Analysis of 2566 IIM cases and 15 651 controls. (B) Analysis of 931 PM cases and 15 651 controls. (C) Analysis of 1360 DM+JDM cases and 15 651 controls. DM, dermatomyositis; IIM, idiopathic inflammatory myopathy; JDM, juvenile dermatomyositis; MHC, major histocompatibility complex; PM, polymyositis.

**Table 2** Loci associated with IIM, PM, DM and JDM cases

Subgroup	Gene Region	Chr	Position	Most Significant SNP	Minor Allele	MAF cases	MAF controls	p Value	OR (95% CI)	Localisation of LD to nearest genes ( $r^2 \geq 0.9$ )	Overlap with other autoimmune diseases
IIM	HLA	6	32 395 726	rs3129843	G	0.24	0.11	$9.14 \times 10^{-133}$	2.74 (2.53 to 2.97)	MHC	Multiple
IIM	PTPN22	1	114 377 568	rs2476601	A	0.12	0.09	$7.22 \times 10^{-9}$	1.32 (1.20 to 1.45)	Exon 12 of PTPN22	ATD, <sup>17</sup> IBD, <sup>16</sup> JIA, <sup>18</sup> RA, <sup>19</sup> SLE, <sup>20</sup> SSC, <sup>21</sup> T1D, <sup>22</sup> VIT, <sup>23</sup>
IIM	YDJC	22	21 985 094	rs5754467	G	0.23	0.20	$4.67 \times 10^{-7}$	1.21 (1.12 to 1.30)	Intron 1 of UBE2L3 complete YDJC	CEL, <sup>6</sup> IBD, <sup>16</sup> JIA, <sup>18</sup> PSO, <sup>24</sup> RA, <sup>19</sup> SLE, <sup>20</sup>
IIM	DGKQ	4	956 047	rs6599390	A	0.30	0.34	$6.48 \times 10^{-7}$	0.85 (0.79 to 0.90)	Intron 21 of DGKQ to intron 2 of SLC26A1 and intron 2 of IDUA	
IIM	STAT4	2	191 917 317	rs4853540	T	0.19	0.22	$1.57 \times 10^{-6}$	0.83 (0.77 to 0.89)	Intron 3 to intron 14 of STAT4	JIA, <sup>18</sup> PBC, <sup>25</sup> RA, <sup>19</sup> SLE, <sup>20</sup>
IIM	MGAT4A	2	99 389 870	rs10189330	T	0.50	0.46	$2.68 \times 10^{-6}$	1.16 (1.09 to 1.23)	Intergenic of C2orf55 (KIAA1211L) and MGAT4A incorporating uncharacterised LOC101 927 070	
IIM	PRR5 L   TRAF6	11	36 492 191	rs570676	A	0.37	0.40	$9.42 \times 10^{-6}$	0.87 (0.82 to 0.92)	Intergenic PRR5L (also known as FLJ14213)   complete TRAF6	
IIM	CCL17	16	57 445 376	rs223900	T	0.28	0.25	$9.97 \times 10^{-6}$	1.17 (1.09 to 1.25)	Intron 1 of CCL17	
IIM	EOMES	3	28 076 283	rs376072	T	0.26	0.29	$1.45 \times 10^{-5}$	0.86 (0.80 to 0.92)	310.68 kb upstream of EOMES	
IIM	CD28	2	204 592 021	rs3116494	G	0.28	0.26	$1.54 \times 10^{-5}$	1.16 (1.09 to 1.24)	Intron 1 to 8.5 kb downstream of CD28	PSC, <sup>26</sup> RA, <sup>19</sup>
IIM	RPL31P10	12	6 523 249	rs11064180	T	0.38	0.41	$1.61 \times 10^{-5}$	0.87 (0.82 to 0.93)	Intergenic of LTBR and CD27	
PM	HLA	6	31 434 366	rs3094013	T	0.25	0.12	$6.36 \times 10^{-76}$	2.97 (2.64 to 3.33)	MHC	Multiple
PM	PTPN22	1	114 377 568	rs2476601	A	0.12	0.09	$7.90 \times 10^{-11}$	1.58 (1.38 to 1.81)	Exon 12 of PTPN22	ATD, <sup>17</sup> IBD, <sup>16</sup> JIA, <sup>18</sup> RA, <sup>19</sup> SLE, <sup>20</sup> SSC, <sup>21</sup> T1D, <sup>22</sup> VIT, <sup>23</sup>
PM	LOC728073  RPL38	17	71 527 243	rs9905921	C	0.48	0.43	$2.01 \times 10^{-6}$	1.26 (1.14 to 1.39)	Intronic (provisional in refseq) of SDK2	
PM	UBE3B MMAB	12	109 980 516	rs7956536	C	0.44	0.46	$3.66 \times 10^{-6}$	0.80 (0.72 to 0.88)	Intron 19 of MYO1H, complete KCTD10 ; UBE3B; MMAB; MVK	
PM	NAB1	2	191 535 576	rs2286896	G	0.15	0.13	$3.76 \times 10^{-6}$	1.35 (1.19 to 1.53)	Complete NAB1	
PM	FAM167A BLK	8	11 333 521	rs17799348	T	0.36	0.39	$4.13 \times 10^{-6}$	0.80 (0.71 to 0.87)	Intergenic FAM167A   BLK	
PM	IL18R1	2	103 012 902	rs1420095	G	0.07	0.09	$6.16 \times 10^{-6}$	0.63 (0.52 to 0.78)	Intron 3 of IL18R1 : 1L18RAP	
PM	SLC26A1 IDUA	4	980 464	rs4690220	G	0.49	0.45	$7.47 \times 10^{-6}$	1.25 (1.13 to 1.37)	2.14 Kb 3' to intron 2 of SLC26A1	SJO, <sup>27</sup>
PM	RGS1	1	192 545 099	rs7535818	G	0.17	0.18	$1.37 \times 10^{-5}$	0.74 (0.65 to 0.85)	25 kb upstream to intron 1 of RGS1	CEL, <sup>6</sup> MS, <sup>28</sup> T1D, <sup>22</sup>
DM+JDM	HLA	6	32 395 726	rs3129843	G	0.21	0.11	$1.72 \times 10^{-48}$	2.18 (1.97 to 2.42)	MHC	Multiple
DM+JDM	ROPN1  ANKRD33B	5	10 517 908	rs4702698	G	0.34	0.30	$4.77 \times 10^{-6}$	1.22 (1.12 to 1.33)	Intergenic of ROPN1L and ANKRD33B	
DM+JDM	PTTG1 ATP10B	5	159 928 876	rs4921293	G	0.39	0.35	$8.27 \times 10^{-6}$	1.21 (1.11 to 1.31)	Intergenic of PTTG1 and ATP10B	
DM+JDM	GSDMB	17	38 066 267	rs1008723	T	0.47	0.49	$9.05 \times 10^{-6}$	1.20 (1.11 to 1.30)	Complete IKZF3, ZBP2 GSDMB	IBD, <sup>16</sup> MS, <sup>28</sup> PBC, <sup>25</sup> RA, <sup>29</sup> T1D, <sup>30</sup>

Analysis of 2566 IIM cases, 931 PM cases, and 1360 DM and JDM cases, compared with 15 651 controls. Loci reported at genome wide significance  $p < 5 \times 10^{-8}$  (bold) or at suggestive significance  $p < 2.25 \times 10^{-5}$ . Coordinates based on GRCh37 assembly. Overlap defined as high LD ( $r^2 > 0.7$ ) between the associations in IIM (including independent effects) and the lead association in another autoimmune disease (as reported on <http://www.immunobase.org>).

ATD, autoimmune thyroid disease; CEL, coeliac disease; Chr, chromosome; DM, dermatomyositis; HLA, human leucocyte antigen; IBD, inflammatory bowel disease; IIM, idiopathic inflammatory myopathy; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; MAF, minor allele frequency; MHC, major histocompatibility complex; MS, multiple sclerosis; PBC, primary biliary cirrhosis; PM, polymyositis; PSC, primary sclerosing cholangitis; PSO, psoriasis; RA, rheumatoid arthritis; SJO, Sjogren's syndrome; SLE, systemic lupus erythematosus; SSC, systemic scleroderma; T1D, type 1 diabetes; VIT, vitiligo.

( $r^2 > 0.7$ ) in other autoimmune diseases are reported in table 2, and the direction of effect is reported in online supplementary table S2.

### Subgroup analysis reveals unique associations within PM and DM

We stratified our cohort by the two largest subgroups within IIM, consisting of 931 adult PM cases (figure 1B and table 2) and 1360 DM cases (figure 1C and table 2). JDM cases were included in the DM analysis to increase power, and on previous evidence that there is not extensive genetic heterogeneity between the subgroups.<sup>4</sup> The only non-HLA region to reach genome-wide significance in either subgroup was *PTPN22* in PM (rs2476601,  $p = 7.9 \times 10^{-11}$ ). Interestingly, with a smaller sample size, the association in PM with *PTPN22* was stronger than in the combined IIM analysis. There was no evidence of association in DM ( $p = 0.19$ ), therefore the stronger effect in PM appears to be driving the association in the combined IIM analysis. Other interesting regions reaching a suggestive level of significance were *SL26A1/IDUA* and *RGS1* in PM, and *GSDMB* in DM (see online supplementary table S3).

### Exonic and eQTL SNPs suggest potential causal variants

Potential functionally relevant variants were investigated for non-synonymous SNPs (table 3) or eQTLs (table 4) that are tagged by the lead SNP ( $r^2 > 0.9$ ). Two variants within the *GSDMB* gene, suggestively associated in DM, are 'potentially damaging' as predicted by PolyPhen-2. The *PTPN22* variant is confirmed to be conserved across vertebrates, as well as an SNP in *UBE3B*. Evidence for eQTLs in cells with immune function (lymphoblastoid cell lines and monocytes) was found in six loci and may help annotate our associations, for example, the association with *NAB1* in PM may be due to an eQTL affecting the expression of *STAT1*, 275Kb upstream.

### HLA imputation confirms alleles of the 8.1 AH as the strongest association in IIM

Due to the complex linkage disequilibrium/haplotype structure in the MHC, interpretation of causal associations and independent effects using SNPs may be inadequate. We used SNP2HLA to impute classical HLA alleles and amino acids from SNP genotyping information. For each analysis, all variants reaching statistical significance ( $p < 6.8 \times 10^{-6}$ ) after each round of conditioning are included in online supplementary tables S4–S15. For many associations, amino acids unique to classical HLA risk alleles were associated at similar levels of significance to the HLA allele. For consistency, four-digit HLA alleles are reported, unless an amino acid is significantly more associated

**Table 4** Evidence of expression quantitative trait loci (eQTL)

Subgroup	Mapped Genes	ImmunoChip lead SNP	eQTL within $r^2 > 0.9$	References
IIM	<i>UBE2L3 YDJC</i>	rs11089637	<i>UBE2L3, RIMBP3</i>	32–33
PM	<i>UBE3B MMAB</i>	rs7956536	<i>KCTD10, MMAB</i>	32–34
PM	<i>RGS1</i>	rs7535818	<i>RGS1</i>	32
PM	<i>NAB1</i>	rs2286896	<i>STAT1</i>	35
DM+JDM	<i>GSDMB</i>	rs1008723	<i>ORMDL3, MED24, KRT222, NR1D1, GSDMB</i>	33–36

eQTLs for the expression of a gene in cells with an immune function (lymphoblastoid and monocytes). RegulomeDB,<sup>37</sup> Genevar,<sup>38</sup> and the Pritchard lab eQTL browser (<http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/>) were interrogated. The most significant SNP or any SNP with  $r^2 \geq 0.9$  with most significant SNP was used. Genes are indicated along with studies in which the eQTLs were reported.

DM, dermatomyositis; IIM, idiopathic inflammatory myopathy; JDM, juvenile dermatomyositis; PM, polymyositis.

than individual HLA alleles. In the combined IIM analysis ( $n = 2566$ ), the most associated variants were classical HLA alleles, with *HLA-DRB1\*03:01* being the most significant four-digit allele ( $p = 2.58 \times 10^{-135}$ , OR = 1.88, 95% CI 1.68 to 2.11). *HLA-DRB1\*03:01* forms part of the 8.1 AH which has been consistently associated with IIM. After conditioning on the effects of *HLA-DRB1\*03:01*, a strong association was found with *HLA-B\*08:01* ( $p = 3.23 \times 10^{-14}$ , OR = 1.58, 95% CI 1.41 to 1.78) suggesting that there is an independent effect within this locus. Further residual associations highlight the heterogeneity within this cohort, so analysis was then conducted on clinical subgroups. Similar associations were found with PM ( $n = 931$ ), *HLA-DRB1\*03:01* being the most significant four-digit allele ( $p = 6.11 \times 10^{-80}$ , OR = 1.99, 95% CI 1.67 to 2.36) and an independent effect with *HLA-B\*08:01* ( $p = 4.17 \times 10^{-9}$ , OR = 1.71, 95% CI 1.43 to 2.05). As the effect size of the HLA is strong in IIM, we hypothesised that we may be able to detect any potential differences between adult DM and JDM, even with a reduced sample size. In adult DM ( $n = 879$ ), *HLA-B\*08:01* was the most significant allele ( $p = 2.46 \times 10^{-42}$ , OR = 1.90, 95% CI 1.66 to 2.17). Conditioning on *HLA-B\*08:01*, there was evidence of multiple independent effects within the *HLA-DQB1* locus, therefore we analysed imputed amino acid residues. Amino acid position 57 of *HLA-DQB1* was more significantly associated with DM than individual *HLA-DQB1* alleles ( $p = 8.95 \times 10^{-14}$ ), with alanine ( $p = 1.29 \times 10^{-12}$ , OR = 1.62, 95% CI 1.44 to 1.83) and serine ( $p = 9.28 \times 10^{-7}$ , OR = 2.15, 95% CI 1.60 to 2.84) conferring the greatest risk. Further association with *HLA-DQB1* remains

**Table 3** Potentially causal exonic SNPs

Subgroup	Mapped genes	Most significant SNP	SNP in LD	SNP location	Amino acid change	PolyPhen-2/SIFT	phastCons17-way
IIM+PM	<i>PTPN22</i>	rs2476601	rs2476601	Non-synonymous coding in <i>PTPN22</i>	R620W	Benign/tolerated	0.99
IIM	<i>DGKQ</i>	rs6599390	rs3796622	Non-synonymous coding <i>SLC26A1</i>	Q556R	Benign/tolerated	0.35
PM	<i>UBE3B MMAB</i>	rs7956536	rs7298565	Non-synonymous coding <i>UBE3B</i>	R346Q	Benign/tolerated	1
			rs9593	Non-synonymous coding in <i>MMAB</i>	M239K	Benign/tolerated	0.17
DM+JDM	<i>GSDMB</i>	rs1008723	rs11078928	Splice acceptor variant in <i>GSDMB</i>			0
			rs2305480	Non-synonymous coding <i>GSDMB</i>	P298S	Possibly damaging/Tolerated	0
			rs2305479	Non-synonymous coding <i>GSDMB</i>	G291R	Probably damaging/Tolerated	0

SNPs investigated for functionality may be the lead association or any SNP with  $r^2 \geq 0.9$ . PolyPhen-2<sup>13</sup> and SIFT,<sup>14</sup> was used to predict the possible impact of an amino acid substitution. phastCons17-way,<sup>15</sup> gives a score of evolutionary conservation in 17 vertebrates, with 1 being the most conserved at that base position.

DM, dermatomyositis; IIM, idiopathic inflammatory myopathy; JDM, juvenile dermatomyositis; PM, polymyositis.

after conditioning, notably an independent effect of *HLA-DQB1\*04:02* ( $p=2.01 \times 10^{-6}$ , OR=1.99, 95% CI 1.52 to 2.58). In the JDM subgroup ( $n=481$ ), *HLA-DRB1\*03:01* was the most associated allele ( $p=7.91 \times 10^{-14}$ , OR=1.90, 95% CI 1.61 to 2.22) and an independent association was observed with *HLA-C\*02:02* ( $p=3.28 \times 10^{-7}$ , OR=1.99, 95% CI 1.55 to 2.52) which is not a part of the 8.1 AH.

## DISCUSSION

This is the largest genetic study to date in IIM, and has revealed several novel suggestive associations in adult and juvenile IIM emphasising the autoimmune architecture of these diseases. We have confirmed *HLA* and *PTPN22* as the most strongly associated regions in IIM, and identified nine additional associations at a suggestive level of significance. Subgroup analysis has identified distinct differences between PM and DM. Identification of exonic and eQTL SNPs has localised association signals to several potential causal variants.

It is reassuring that associations such as *PTPN22*, *STAT4* and *UBE2L3* follow a similar genetic profile as reported in other autoimmune diseases. The most significantly associated SNP in the *PTPN22* region is the rs2476601 variant, a C>T polymorphism that results in a non-synonymous arginine (R) to tryptophan (W) amino acid change at position 620. Although this SNP has been extensively studied in the context of autoimmunity, there is no consensus regarding the functional consequences of this SNP. Some studies report a gain of function mutation by enhancing the inhibitory effect on T-cell receptor (TCR) signalling,<sup>39</sup> while others report a loss of function by increased degradation of the protein and a diminished inhibitory effect on T cell activation.<sup>40</sup>

*STAT4* is an important transcription factor for many genes involved in T cell differentiation and has previously been associated with DM in the Japanese population.<sup>41</sup> Stepwise logistic regression analysis was conducted on all regions in this study; however *STAT4* is the only locus with evidence of independent associations. The three independent SNPs are in LD with associations in different diseases. The lead SNP in *STAT4* is protective, and in moderate LD with protective SNPs in *STAT4* reported in inflammatory bowel disease, Crohn's disease and ulcerative colitis.<sup>16</sup> The independent risk effect of rs10174238 is the same SNP reported in juvenile idiopathic arthritis,<sup>18</sup> and is in strong LD with disease-associated SNPs in rheumatoid arthritis and systemic lupus erythematosus.<sup>19 42</sup> A SNP in high LD with rs932169 has been reported to be associated with primary biliary cirrhosis.<sup>25</sup>

The most significantly associated SNP in the *YDJC* gene tags an established autoimmune susceptibility locus where *UBE2L3* is thought to be the causal gene.<sup>43</sup> This risk haplotype is thought to increase *UBE2L3* expression in B cells and monocytes and amplify nuclear factor kappa-B (NF- $\kappa$ B) activation.<sup>43</sup>

Stratification by clinical subgroup revealed further novel suggestive associations. These distinct differences between PM and DM suggest different autoimmune pathways in these subsets of IIM. For example, when splitting the total IIM cohort into PM and DM, we have shown that the association with *PTPN22* is predominantly driven by a strong association with PM. For all associations, we have stratified by clinical subgroup and reported the summary statistics in online supplementary table S3. Patients with IBM were included in the combined IIM analysis on the basis of their diagnosis as an inflammatory autoimmune myopathy, however we did not analyse this subgroup separately due to a lack of power ( $n=252$ ). Removing this group from our analyses did not make any substantial difference in associated

regions, however the strength of the signals were attenuated in most instances. With eight non-HLA loci reaching our level of suggestive significance in PM, and only three in DM, it may be that the Immunochip is explaining less of the genetic risk to DM. This may be due to lack of power, the selected content of the Immunochip, heterogeneity of phenotypes within DM or a weaker genetic influence compared with other autoimmune diseases. Some previous reported associations with DM failed to replicate in this study. We looked for evidence of association with loci that have previously been associated with IIM that did not reach our suggestive level of significance. For example, in the DM and JDM subgroup analysis, an association was found with rs2618476 ( $p=3.2 \times 10^{-5}$ , OR=1.2, 95% CI 1.1 to 1.32), an SNP in B lymphoid tyrosine kinase (*BLK*). rs2618476 is a proxy for an SNP that was associated with DM in the Japanese population,<sup>44</sup> and is also highly correlated ( $r^2 > 0.8$ ) with associations found in systemic lupus erythematosus and rheumatoid arthritis.<sup>20 29</sup> With this knowledge, this association becomes more convincing, whereas the single association in the *FAM167A-BLK* region in the PM subgroup (rs17799348) that is independent of the established risk haplotype, is less so.

It is important to note that this study was conducted on Caucasian IIM individuals. While there is evidence that risk loci may be shared across populations, such as *STAT4* and *BLK* in the Japanese population, the association between *PTPN22* and autoimmune disease is unique to Caucasians as the R620W variant is rarely seen in Asian populations. There is therefore a need to conduct further genetic studies on different IIM populations.

The Immunochip contains a dense set of SNPs covering 186 loci based on evidence of association with 12 different autoimmune and inflammatory diseases.<sup>18</sup> IIM was not one of these diseases, so this study can be seen as an exploratory investigation to assess genetic overlap with other autoimmune diseases, rather than the identification of genes novel to IIM. With 2566 samples, Immunochip studies of similar size have revealed multiple non-HLA associations reaching genome-wide significance.<sup>18 45</sup> The fact that only a single locus reached this threshold may be due to low statistical power owing to phenotypic heterogeneity within IIM. A more conservative level of significance ( $p < 2.25 \times 10^{-5}$ ) revealed suggestive associations of interest. SNPs that are the same, or in high LD with established autoimmune variants, along with biological knowledge and/or evidence of functionality may lead us to pursue these associations with more confidence. Indeed, a recent Immunochip study in type 1 diabetes calculated a Bayesian posterior probability of disease association  $> 0.9$  of SNPs reaching a suggestive level of significance ( $p < 1 \times 10^{-5}$ ) when there is evidence of genome-wide significance in other Immunochip studies.<sup>30</sup>

Due to the extended haplotypes that are present in the HLA region, for many associations, alleles carried on the same haplotype reached an equivalent significance level. For consistency, the most associated allele was used in the stepwise conditional analysis; however, this is not to say that the allele is causative. Interestingly in PM, two alleles frequently inherited together on the 8.1 AH (*HLA-DRB1\*03:01* and *HLA-B\*08:01*) show evidence of having independent effects. This may also be the case in DM, however, after conditioning on *HLA-B\*08:01*, the association with other alleles of the 8.1 AH did not reach genome-wide significance. In DM, the independent association with amino acid position 57 in *HLA-DQB1* may explain part of the risk within this gene. Indeed, this position is an established risk factor for type 1 diabetes.<sup>46</sup> In this study, classical four-digit HLA alleles were preferentially reported, unless an amino acid

association explained more risk than HLA alleles alone. However looking at amino acids may give insight into functionality. For example, the association with *HLA-DRB1\*03:01* may be explained by the presence of amino acids that are unique to that allele. An asparagine at position 77, and an arginine at position 74 also were highly associated with IIM (see online supplementary table S4), and these residues are predominantly found on *DRB1\*03* alleles. As there are multiple residues unique to this allele, it is hard to tease out which positions may be functionally important; however the location of these amino acids in the *HLA-DRB1* molecule may give insight (see online supplementary figure S4). Amino acid position 74 of *HLA-DRB1* lies within the peptide binding groove, and almost all of the risk at this position can be explained by the presence of an arginine ( $p=3.1 \times 10^{-72}$ , OR=2.83, 95% CI 2.53 to 3.17). The location of Arg-74 may change the structure of the peptide binding groove in such a way as to accommodate autoantigenic peptides. Indeed Arg-74 is an established risk factor for the development of autoimmune diseases.<sup>47</sup> A similar phenomenon is seen with *HLA-B\*08:01* and the occurrence of Phe-67 and Asp-9 (see online supplementary figure S5).

Alleles of the 8.1 AH have frequently been associated with the presence of myositis autoantibodies such as anti-Jo-1 and anti-PM-Scl. It may be that the association with the 8.1 AH and IIM is due to the prevalence of these antibodies, and weak associations with other *HLA*-alleles may be due to associations with autoantibodies that are less frequent in the disease subgroup. Further work is planned to stratify patients by serotype to clarify these differences.

This study has revealed new suggestive associations with IIM in the Caucasian population, and independent associations with PM and DM, and has shown that subgrouping patients into clinical subgroups is important to expand our knowledge of IIM. This international collaboration has made it possible to perform the largest study to date in IIM and it has considerably expanded our knowledge about the genetic architecture of this rare disease.

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